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NOTES ON THE ORIGIN OF THE LIVER.

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A. *Preliminary.*

In many invertebrates there is found in connection with the mesenteron an organ which has received the name of "liver;" in others, some of the endodermal lining cells of the gut are different from the rest, and have been regarded as "hepatic." It is much open to question whether any of these structures are either physiologically or morphologically equivalent to the organ known as liver in the Vertebrata. It is best, for the present, to speak of these structures as the "glands of the mid-gut," or "glands of the mesenteron." An investigation of these "glands of the mesenteron," with a view to determine their relationships to the liver of vertebrates, is one which may lead to important results. Such a research may be made from (*a*) the physiological side, or (*b*) the morphological. An inquiry from the latter point of view will include—(1) an examination of the structure of the liver of as many types of vertebrates as possible, and the small liver of lower forms particularly should be investigated, so as to learn in its simplest condition the plan of structure of the organ in this large group; (2) a comparison of this structure with that of the "glands of the mesenteron" in as large a number of diversified types of invertebrates as possible; (3) a study of the ontogeny of the liver of Vertebrata; (4) a comparison of the facts thus learnt, with the development of the glands of the mesenteron in invertebrates.

From the nature of the secretion produced by the so-called "livers" of invertebrates, and from the digestive actions of these secretions, some observers have classed these organs as "pancreatic" rather than "hepatic." Writing on the intestine of Decapods and its gland, Cattaneo[1] finds that the gland of

[1] *Atti. Soc. Ital. Sci. Nat.*, xxx., 1887, p. 238.

the mid-gut or "liver" is complex enough, in function, to be compared to that of all the vertebrate's digestive glands together—in other words, he finds it to be "poly-enzymatic." By some it has been called "hepato-pancreas." A. B. Griffiths[1] finds that the "liver" of *Carcinus mœnas* is in function pancreatic. He also concludes that the secretion of the so-called "hepatic cells" in Araneina[2] is similar to the pancreatic fluid of Vertebrata both in function and in chemical nature.

In July 1889, I published, in conjunction with my colleague, Dr Lewis Jones,[3] an account of the minute structure of the liver of a large number of vertebrated types, but particularly with the object in view of explaining some doubtful points as to the liver of mammals, and of showing the relation between the well-known tubular liver of lower vertebrates, and the apparently parenchymatous arrangement found in this organ in the mammal. In this paper we showed that the liver of all the vertebrates examined is built up on the same plan, and that in all cases the organ is a tubular gland, but one having a special and unique feature, not found in any of the ordinary tubular glands, such as the pancreas or salivary glands. The vertebrate's liver is in every case composed of an anastomosing network of cylinders of cells. These cells are arranged in a single layer, so as to form the boundary walls of a network of anastomosing "secretion channels" of minute calibre, a secretion channel penetrating the axis of each cell-cylinder, branching with it and following its anastomoses. The number of cells seen in a cross section of one of these cylinders, and disposed around the central lumen, varies from two to six, being generally more numerous in the livers of lower types. The meshes of the network formed by the branching and anastomosis of these cylinders are in some cases broad, in others narrow, varying as the meshwork of secretion tubes is loose or close. These meshes are occupied by blood "capillaries," having walls composed of a single layer of flattened nucleated epithelial cells, and which are themselves arranged as a network. Each mesh of the blood-capillary system is thus occupied by a secretion tube, as each loop in the system

[1] *Proc. Roy. Soc. Edin.*, xvi., 1888–9, p. 178.
[2] *Proc. Roy. Soc. Edin.*, xv., 1887–8, p. 111.
[3] *Journal of Physiology*, vol. x., No. 5, p. 408, 1889.

of secretion cylinders is filled by a blood capillary. There is a further characteristic feature in the liver structure as compared with that of an ordinary tubular gland, which is of very great importance. The cells forming the walls of the secretion tubes *are not surrounded by any basement membrane* at all comparable to the layer so named in the case of a compound tubular gland of the ordinary type. The external surfaces of the liver cells are therefore in contact with the thin walls of the blood capillaries, and every proper secretion cell has on one side of it a blood channel with a definite wall, and on the other a secretion channel. Although this arrangement is not very obvious in the livers of the higher vertebrates, it can nevertheless, in a properly prepared section, be made out. A section of one of the livers in which the structure above summarised is most obvious, presents an appearance at first sight very like that of a section of a salivary gland; a little examination, however, will show the following two important features of difference:—(1) *That in the liver the secretion tubes form an anastomosing network;* (2) *that no basement membrane is present. In no vertebrated animal which I have examined is there to be found a tubular structure without anastomosis.*

Although the general plan of structure of the liver is the same in all the Vertebrata, there are, of course, differences between the various types examined. These differences seem to be (*a*) differences of degree of penetration of blood-vessels amongst the cell-cylinders; (*b*) differences in the density of the network of liver tubes; (*c*) differences as to the number of rows of cells to form the walls of the secretion channels; (*d*) the presence in mammals of lobules, which have probably arisen by a tendency for blood capillaries to arrange themselves around foci of exit for blood; (*e*) the relative number of fine ducts in the liver substance depending upon the degree to which the bile duct has extended within the organ; (*f*) the relative amount of connective tissue extending along the course of the bill ducts and portal vein branches, so as to form a definite Glisson's capsule.

An anastomosing network of the kind here briefly described can only have arisen in one of two ways—(*a*) by the union together of the extremities of originally distinct tubes; (*b*) by

the subdivision of an originally solid mass of cells, owing to the development within it of a network of intercellular spaces. For reasons which I have detailed and discussed in the *Journal of Physiology*, I and my colleague have put forward the latter hypothesis. On this theory the liver of the Vertebrata is believed to have been evolved by some such steps as the following[1]:—

"1. The formation from the gut of a hollow diverticulum constituting at first a simple tubular gland, lined by specially modified secreting endoderm. This stage is represented in actual anatomy by the simple hepatic diverticulum of the *Amphioxus*, and is reproduced in the ontogeny of the higher vertebrates by the formation of the primary hepatic outgrowth of the hypoblast, as in the Chick, &c.

"2. The copious subdivision of the endoderm cells at the cæcal extremity of this diverticulum to form a solid mass of cells, slightly penetrated by minute channels for escape of secretion, the original diverticulum becoming a duct.

"3. The further multiplication of the cells of this mass, and its penetration by blood-vessels, so as imperfectly to divide it into solid anastomosing rods of secreting cells drained by a system of intercellular bile canals. This step is probably preserved in the liver of the lamprey.

"4. The more complete penetration of blood-vessels between the cylinders of hepatic cells so as to form a well-marked system of broad blood channels, separating a network of rods of cells which come to be arranged in a single layer around bile capillaries. This is seen in the permanent condition of fishes, amphibians, and reptiles, and is ontogenetically repeated in the development of the mammal.

"5. A still finer penetration by blood-vessels and further subdivision of the hepatic cylinders, associated with an arrangement of the blood capillaries around foci of exit for blood, so as to form hepatic lobules. This is the condition found in the adult Mammalia."

It will be at once seen that this view of the evolution of the vertebrate's liver implies that the organ has arisen within the limits of the group *Vertebrata*, and that the structures supposed to be of "hepatic" nature in invertebrates have no connection with it, and are not phylogenetically related to it. In putting forward this view, I and my colleague were guided almost entirely by the facts we learn from an examination of the structure of the liver of adult vertebrates. It is clear, however, that the conclusions must be tested by an investigation of the "hepatic" organs of invertebrates, and by a study of the ontogeny of the organs which are named "liver" in both

[1] Quoted from the *Journal of Physiology*, vol. x. p. 425.

vertebrates and invertebrates. I have begun a research on this question, using ordinary histological methods. My material is chiefly hardened in some chromium preparation, or in picric acid, or in alcohol; this is followed by staining in bulk, embedding in paraffin, and cutting with the rocking microtome. In some cases I fail to obtain good results by these methods; and have found fixing in osmic acid to be very useful in some cases. In studying the ontogeny of the liver I have at present worked chiefly with frog, chick, and rabbit embryos, ordinary embryological methods being employed. Most of my sections are about 10μ in thickness. Some points of interest have already arisen in my work, and these form the subject of the present notes. I will first briefly describe the structure of the "gland of the mesenteron," in a few typical invertebrates, and after indicating some points on the development of the liver in vertebrates, will discuss the conclusions which the facts published in the *Journal of Physiology*, together with those now described, seem to indicate.

B. *Structure of the "Liver" of some Invertebrata.*

1. *Mollusca.*—In this phylum there is almost always present some appendage or appendages of the mesenteron, to which the term "liver" has been applied. They take the form of more or less wide sacculi or outgrowths from the endoderm of the gut, which, becoming drawn out and subdivided, form a number of cæcal acini. These may be grouped into larger or smaller lobes, held together by connective tissue, so as to form, in some cases, organs of considerable magnitude. In most of the *Lamellibranchiata* the gland of the mesenteron is well developed, forming a number of lobes opening into the "stomach" by several wide pouches or ducts. In the most typical of the *Gasteropoda*, it is a largely developed organ, occupying a considerable portion of the visceral dome; it surrounds the coils of the intestine, is arranged into lobes, and opens by one or more ducts into either the intestine or the "gastric" enlargement. In some Mollusca, viz., the so-called *Nudibranchiata*, the glands of the mesenteron consist of large metamerically repeated pairs of tube-like diverticula of the gut, from which numerous glandular outgrowths are given off; and

in some cases these divertieula send eæeal prolongations into the eirrhus-like processes of the dorsal integument—a fact whieh snggests that they may have some function other than that of secretion of a digestive fluid. Fol,[1] in deseribing the microscopic anatomy of *Dentalium*, states that the "liver" is a collection of eæea, which open into the stomach. The cæca of the "liver" are lined by an epithelium widely differing from that of the stomach, though a transition from the one to the other is found, so that it is difficult to mark the boundary between stomaeh and liver duct. The protoplasm of the "hepatie" eells has a spongy natnre, and in seetions appears to be honey-combed into a number of large spaees. The cells are deseribed as narrow and rather elongated.

The "liver" of *Mya arenaria* is seen microseopieally to be composed of a large number of wide cæca, mostly oval or rounded in form, and grouped together upon branches of broad divertieula from the stomaeh. These cæca are loosely bound together by conneetive tissue into lobes, and their general features, as seen in a seetion, are shown in Plate IV. fig. 1. Eaeh ultimate pouch or cæeum is formed of—(*a*) a wall of condensed connective tissue, eontaining small oval nuclei, *i.e.*, a basement membrane, eontinuous with the similar membrane upon whieh the epithelial cells lining the stomaeh are placed; (*b*) a layer of much modified epithelial eells continuous with those of the general endoderm. The protoplasm of these eells is dense, and stains readily near the basement membrane; but more towards the eentres of the eæea the protoplasm of the eells seems to be eonfluent into a general network of finely granular fibrils, anastomosing around large clear spaces, whieh are gradually lost in the large central eavities of the eæca. The inner contours of the eells are therefore very indefinite, and their protoplasmic bodies are, in faet, deeply honey-combed, as is described by Fol, in *Dentalium*. The nuelei of the "liver-cells" are round, and lie in the deeply-stained denser peripheral protoplasm. Eaeh nueleus contains a marked nucleolus. Groups of the ultimate pouches may readily be seen in sections uniting together into common larger saeeuli, the walls and epithelium of whieh have the same characters as are

[1] *Arch. Zool. Expér. et Gen.*, vii., 1889, p. 91.

found in the ultimate pouches. The epithelium of the "liver" cæca is very different in character from that forming the endodermal lining of the stomach, the cells of which are elongated in form, and richly ciliated. By examination of sections with a low power it is clear that there is *no anastomosis between different cæca.*

In all essential particulars the gland of the mesenteron in *Anodonta cygnæa* agrees with that of *Mya.* There is no trace of any anastomosis of cæca with each other. The cells forming the proper secreting substance are planted upon a definite basement membrane, and are honey-combed in their central parts as in *Mya.* The "liver" of *Helix pomatia* is built up on the same plan as that of *Mya* and *Anodonta*, viz., isolated cæca, made up of a basement membrane, lined by honey-combed cells. The whole texture of the organ is, however, denser, and the outlines of the pouches are more irregular than in *Anodonta* and *Mya.* The "liver" cæca join the stomach and intestine at several points, and in a fortunate section the transition from the elongated columnar epithelium of the intestine to the honey-combed cells of the "liver" cæca may be traced along a short duct. In some cases in *Helix*, viz., in the "liver" of a hibernating animal, the spaces in the honey-combed part of the protoplasm are found to be occupied by rounded masses of a dark brown colour, and with a highly refractive appearance.

In *Limax hortensis*, as would be expected, the same general characters are found. The tissue is looser than in *Helix*, and the minute characters of the cells are somewhat different from those already described. The peripheral portions of the cells contain nuclei, and stain deeply, as in *Mya* and *Anodonta*, but the central portions are not deeply honey-combed. The inner margins of the cells immediately bounding the cavities of the cæca are, therefore, more definite. The whole of the central portions of the protoplasm, however, stain lightly, and are loaded with a dark brown granular substance. The nuclei of the cells are always large and distinct.

The characters of the "liver" in *Aplysia* are very similar to those of *Limax.* The same general plan as in other Mollusca is present. The outlines of the cells lining the cæca are definite,

and they are not honey-combed. The cells vary much in shape, but are mostly narrow and elongated, and are somewhat swollen at their free ends, having pyriform or rounded inner extremities.

2. *Arthropoda.*—In the great majority of the Crustacea, some sort of gland of the mesenteron is present. In most of the Entomostraca a pair of short rounded wide cæca are given off from the anterior part of the mid-gut. This is the case, for example, in *Daphnia* and *Lepas*. In these cases, the walls of the cæca do not appear to be very different from the rest of the lining of the mesenteron, though they are probably the first rudiments of what, in other cases, becomes a considerable gland. In some of the Entomostraca these cæca are branched and glandular in appearance, and in any case, whether branched or not, generally lie in the cephalic region. In the Hedrophthalmia division of the Malacostraca, cæca are found in connection with the anterior part of the mid-gut, as in the Entomostraca, but they are generally long unbranched tubes which pass backwards into the thoracic or abdominal regions. They are well formed in *Oniscus*, where each diverticulum is branched near its origin, so that we find two pairs of long glandular tubes. In *Gammarus*, a pair of similar tubes are found passing backwards beneath the gut. In the Schizopoda division of the Podophthalmia, similar long unbranched cæcal tubes are found. In the Decapoda, however, the cæca, though developed from the same part of the mesenteron, are much branched into a number of straight finger-like tubes loosely connected together, and grouped into lobes on the sides of branches of a pair of ducts, altogether forming a pair of large glandular organs. This is the case with the so-called "liver" of *Astacus*, *Carcinus*, *Homarus*, &c. In some of the Crustacea a pair of cæca given off from the anterior part of the mid-gut are not found, but in place of them functionally are a number of more or less metamerically arranged pairs of outgrowths from the rest of the mid-gut. In the Arachnida, there are frequently present one, two, three, or more pairs of tubular organs opening into the mid-gut, and bearing tufts of glandular cæca. (These must not be confounded with the pairs of gastric cæca found in spiders, and which are not formed from the mid-gut, nor with the Malpighian tubules developed more posteriorly, and which are

undoubtedly of "renal" nature.) In the Inseeta "hepatie" divertieula are not frequently found, but the so-ealled "pyloric eæea," of sueh a type as *Periplaneta*, may be of this nature. The most highly developed of all the glands of the mid-gut amongst the Arthropoda is the "hepato-panereas" or "liver" of the Deeapoda, *e.g.*, *Astacus fluviatilis*, and a short description of it will suffiee for the purposes of this paper. The "liver" of *Astacus* is well known as forming a pair of yellow eoloured glands made up of several loose lobes lying at the sides of the gut, and posterior parts of the "stomaeh." They are eomposed of a large number of long proeesses grouped together on branehes of the "hepatie" duets. The whole texture of the gland is very loose, and the general arrangement of its strueture ean be well seen without mieroseopie assistanee. On mieroseopie examination of sections, eaeh finger-like process is found to be a eæenm, having a thin wall of conneetive tissue lined internally by a single layer of large eells. A seetion of one of the cæca is figured in Plate IV. fig. 2. The eells are narrow and elongated, and stain well. Their nuclei are found in the peripheral parts where the protoplasm is dense. More internally, the body of each cell is found to be mueh vaeuolated, and its texture spongy, the protoplasm again beeoming dense at the free inner borders of the cells, where a deeply stained band is found forming the immediate boundary of the large eentral eavity. The cells are particularly narrow at their bases where the nuclei lie, and are broader, with rounded margins, at their central free extremities. They are thus more or less elub-shaped, and the border of the eentral cavity is in eonsequenee not eircular, but sinuous. When the extremity of one of the eæca has been eut longitudinally in the seetion, there is found to be a dense mass of deeply vacuolated eells completely filling up the eavity of the tube in this region.

On comparing the most highly developed "liver" of the Arthropoda with the most highly developed one of the Mollusca, there is a general agreement as to strueture, and in the eommon feature of being markedly different from the liver of Vertebrata. The chief difference is in the form of the ultimate cæca or aeini of the gland—in Mollusca they are rounded, short, and pouch-like, in Arthropoda, elongated and tube-shaped.

3. *Lower Groups.*—In the Vermes, generally speaking, separate glands are not developed in connection with the midgut. In some cases, however, the epithelium of this part of the alimentary canal is found to have a different character to that of the other parts of the tube. The cells in these cases are swollen, more granular, and have brownish or yellowish contents. In some of the Trematodes, *e.g.*, *Distomum*, and in Turbellarians, *e.g.*, *Planaria*, the mesenteron is thrown into cæcal branches, the cells lining which have more "glandular" characters than those forming the rest of the endoderm. The cæca of the intestine of *Distomum* are pouches or branched tubular outgrowths, and are composed of a thick basement membrane lined by a single layer of elongated cells, whose nuclei are placed at about their centres. The peripheral parts of the cells are striated longitudinally, and the central portions are softer, more irregular, and finely granular. These cæcal branches are probably secretory, and may be regarded as appendages differentiated for the purpose of producing a digestive fluid. In some Chætopods, we find the specially differentiated "hepatic" cells along the whole length of the intestine, as in *Lumbricus*. In some of the higher Chætopods, *e.g.*, *Aphrodita*, it is well known that the mesenteron gives off definite cæca, each terminated by what appears to be a secreting gland. These cæca are generally regarded as "hepatic." In the Rotifera, and again in some Polyzoa, the epithelium of the mesenteron has an appearance suggestive of secretory functions.

So far as my observations have at present extended, the so-called "liver" of invertebrated animals has in no case a structure at all comparable to that of a vertebrate. In the former, there is never present that anastomotic network of liver-cylinders or liver-tubules, which is so characteristic of the latter; nor is there to be found in the invertebrate any such penetration of the gland substance by a network of blood channels as is so striking a feature of the vertebrate liver. The secreting structures in the invertebrate's liver, on the contrary, are large cæcal branches or saccular dilatations of a duct, and have a relatively large cavity surrounded by definite walls formed of the secreting cells, *planted upon a basement membrane of connective tissue*. As far as my

researches extend, this is never the case in the liver of a vertebrate.

We are thus led to conclude—*that the "liver" of Invertebrata differs fundamentally in structure from that of Vertebrata;* (a) *in the absence of an anastomosing system of secretion tubules arranged so as to form a network;* (b) *in the absence of any co-existing network of blood-vessels;* (c) *in being composed of cæcal dilatations of a duct, consisting of secreting cells arranged around large central cavities; and* (d) *in the presence of a basement membrane around the cells.*

This conclusion being granted, we next have to inquire whether the vertebrate's liver has been evolved from one of the invertebrate type. It can only be conceived possible for this to have occurred if it be granted that union together of the cæcal extremities of originally distinct tubules, so as to form an anastomosing network, can take place. If this is allowed, it is easy to understand how, by repeated branching, and by the recurrence of anastomosis at the ends of contiguous branches, a network of any degree of fineness may have arisen. I find it difficult, however, to believe that this has been the case, partly on account of the presence of a basement membrane in the invertebrate's liver, and its absence in that of vertebrates, and partly for reasons which Lewis Jones and I have indicated in our paper in the *Journal of Physiology*, to which the reader is referred.

We must, therefore, in the present state of our knowledge, conclude that the liver of Vertebrata *has not been evolved from that of any known invertebrate type, and must therefore have arisen within the limits of the vertebrate phylum itself.*

This conclusion is supported by the fact that no transitional conditions between the two kinds of "livers" are known. It is also strengthened by the consideration that in the development of the vertebrate liver no condition is found at any stage which could properly be compared to the liver of any invertebrate. Moreover, during its development, in the Chick for example, it is not proved that the original cell cylinders are ever without anastomosis, though this is generally said to be the case. As a further point tending to uphold this conclusion, is the fact that the functions performed by the "liver" in the two cases are, as

far as we know, widely different—that of vertebrates seldom, if ever, secretes a digestive fluid *containing a ferment*, whilst that of the invertebrate is known, in several cases, to be "polyenzymatic."

All the facts of the case, then, tend to show that the summary of the stages in the evolution of the vertebrate liver, which is quoted at page 169 of this paper, is, in the present state of our knowledge, correct. Having thus concluded that the vertebrate liver was evolved within the limits of the vertebrate phylum we next have to inquire: In relation to the performance of what function was the vertebrate liver evolved? What circumstances led to its origin? The first step in this inquiry is a study of the actual development of the liver in Vertebrata.

C. *Development of the Liver of some Vertebrated Types.*

Before entering upon a description of my own observations on this part of the subject, it will be well if I briefly allude to what is currently taught, and to some of the points which others have described as the development of this organ in vertebrates.

In Elasmobranch fishes, Balfour[1] found that the middle part of the gut remains till late in embryonic life in connection with the yolk sack by the vitelline canal, which canal opens into the gut *immediately behind* the entrance of the hepatic duct. As to the relations of the blood-vessels to the developing liver, Balfour[2] says—"On the formation of the liver the proximal end of the subintestinal vein becomes the portal vein, and it is joined as it enters the liver by the venous trunk from the yolk sack."

The formation of the gut in the Teleostei is not very well known, and the origin of the liver in them is therefore somewhat obscure. Balfour[3] found that "in the just-hatched larva of an undetermined fresh-water fish, with a very small yolk sack, the yolk extended along the ventral side of the embryo from almost the mouth to the end of the gut. The gut had, except in the hinder part, the form of a solid cord resting in a concavity of the yolk. In the hinder part of the gut a lumen was present, and below this part the amount of yolk was small."[4]

[1] *Comparative Embryology*, 1st ed., vol. ii. p. 45.

[2] *Ibid.*, vol. ii. p. 53.

[3] *Ibid.*, vol. ii. p. 61.

[4] I shall endeavour to show in the sequel what importance is to be attached to these facts.

As to the development of the liver in Teleostei, Balfour[1] says :—

"The liver in the earliest stage in which I have met with it in the trout (twenty-seven days after impregnation) is a solid ventral diverticulum of the intestine which in the region of the liver is itself without a lumen." Referring to the relation of the liver duct to the yolk sack, Von Baër states that the yolk sack remains "in communication with the intestine immediately behind the liver," whilst on the other hand Lereboullet[2] finds "that there is a vitelline pellicle opening *between the stomach and the liver*, which persists until the absorption of the yolk sack." The relations of the liver to blood-vessels in Teleosteans also appear to be interesting. Rathke[3] and Lereboullet[4] say that the subintestinal vein at first *breaks up into lacunæ of the yolk sack, from which the blood is carried direct to the heart.* Later on, when the liver is developed, the subintestinal vessel breaks up into capillaries in this organ, thence passes to the yolk sack, and then to the heart. They also find that an artery is given off from the aorta, penetrates the liver and there breaks up into capillaries continuous with those of the yolk sack.

In the course of the development of the Cyclostomi, the alimentary canal immediately behind the stomach "dilates considerably and on the ventral side is placed the opening of a single large sack, which forms the commencement of the liver. The walls of the hepatic sack are posteriorly united to the yolk cells." The subsequent history of this hepatic sack is thus summarised by Balfour :—"The primitive hepatic diverticulum rapidly sprouts out and forms a tubular gland. The opening into the duodenum changes from a ventral to a lateral or even dorsal position. The duct leads into a gall bladder imbedded in the substance of the liver. Ventrally the liver is united with the abdominal wall, but laterally passages are left by which the pericardial and body-cavities continue to communicate." The subintestinal vein in Cylostomi has the same relations to the liver as are found in Elasmobranchs.

In the Ganoids, the relations of the liver, stomach, and yolk sack are very different from those found in other fishes. Balfour[5] says—"In most Vertebrata the yolk cells form a protuberance of the part of the alimentary canal immediately behind the duodenum. The yolk may either, as in the Lamprey or Frog, form a simple thickening of the alimentary wall in this region or it may constitute a well-developed yolk sack, as in Elasmobranchs and the Amniota. In either case, the liver is placed *in front* of the yolk. In the Sturgeon, on the contrary, the yolk is placed almost entirely *in front of the liver*, and the Sturgeon appears to be also peculiar in that the yolk, instead of constituting an appendage of the alimentary tract, *is completely enclosed in a dilated portion of the tract, which becomes the stomach*

[1] *Op. cit.*, vol. ii. p. 63.
[2] Quoted from Balfour, *op. cit.*, vol. ii. p. 65.
[3] For reference *vide* Balfour, *op. cit.*, vol. ii. p. 67.
[4] For reference *vide* Balfour, *op. cit.*, vol. ii. p. 66.
[5] *Op. cit.*, vol. ii. p. 90.

Behind the stomach is placed the liver. The subintestinal vein bringing back the blood to the liver appears to have the same course as in the Teleostei, in that the blood after passing through the liver is distributed to the walls of the stomach (*i.e.*, the yolk), and is again collected into a venous trunk which falls into the sinus venosus. As the yolk becomes absorbed, the liver grows forwards underneath the stomach, till it comes in close contact with the heart." On the other hand, in *Lepidosteus* there is a large yolk sack opening by a narrow vitelline duct into the intestine, immediately behind the liver.

In the Amphibia, the liver begins as a ventral diverticulum of the gut immediately in front of the yolk.

It is generally taught that the first rudiment of the liver in the Amniota, consists of one or two ventral diverticula from the gut, one or both of which grow into a special thickening of the splanchnic mesoblast. "From[1] the primitive diverticula there are soon given off a number of hollow buds which rapidly increase in length and number and form the so-called hepatic cylinders. They soon anastomose and unite together and so constitute an irregular network. Coincidently with the formation of the hepatic network, the united vitelline and visceral vein or veins in their passage through the liver give off numerous branches, and gradually break up into a plexus of channels which form a secondary network amongst the hepatic cylinders."

1. *Development of the Liver in the Frog.*—The early stages in the formation of that part of the gut from which the liver develops must first be briefly described. At the close of segmentation, the frog's ovum consists of two unequal parts, viz., a mass of small epiblastic cells at one pole, and an accumulation of larger or yolk cells at the other pole, with a segmentation cavity between them. At an early stage, the epiblastic cells begin to extend on all sides around the yolk cells, so that the line of junction of the two kinds of cells seen on the surface of the sphere gradually becomes nearer and nearer to the yolk-pole. Very soon, there takes place on that side of the ovum, which will subsquently become the dorsal, an inflection of the epiblast cells along a small arc of the epiblastic margin. This is the commencement of an asymmetrical invagination. The cells which thus become inflected are soon found, on examining a section, to form two strata: (*a*) a single layer of hypoblast; (*b*) several layers of mesoblast-cells lying between the superficial epiblast and the deepest stratum of the inflected cells. Gradually, as the epiblast grows more and more

[1] Balfour, *op. cit.*, vol. ii. p. 632.

over the yolk cells, the extent of the arc of inflection becomes greater till the whole of the epiblastic margin is inflected. The circle representing this involuted margin is the blastopore, which, so far as my observations extend, appears subsequently to become the anus.[1] The proportion of the hypoblastic layer which is formed from the epiblastic cells actually inflected is small, the greater part of it being produced by a transformation of yolk cells into hypoblast in a line with those cells which are actually invaginated. This transformation of yolk cells into hypoblast extends most rapidly along what will be the dorsal wall of the gut. Meanwhile, a cavity (the mesenteron) has been formed between the extending hypoblast and the underlying yolk cells. In a short time, the hypoblast extends laterally, in what will form the anterior part of the embryo, so that this part of the mesenteron gradually becomes completely surrounded with a true hypoblastic epithelium. This lateral extension of the hypoblast is solely due to a transformation of yolk cells. The cavity of the mesenteron becomes similarly completed posteriorly, but in its middle regions the lateral transformation of the yolk into hypoblast goes on but slowly. It thus follows that the floor of the middle part of the mesenteron is formed only of an extensive mass of yolk cells. This can be well seen in sections across the middle of the frog's embryo about six days after impregnation. Plate IV. fig 3, is a drawing of such a section, and the lateral continuity of the hypoblast with the ventral yolk cells is well shown. The first rudiment of the liver is found in sections of a frog's embryo of about seven days from impregnation. In its earliest stage, it consists of a ventral prolongation of the gut into the anterior part of the mass of yolk cells. The cells immediately bounding the cavity of this diverticulum are yolk cells rapidly becoming converted into hypoblast. This ventral prolongation of the gut, which we may call the primary hepatic outgrowth, passes in a direction ventralwards and forwards towards the anterior end of the yolk mass. In Plate IV. fig. 4, a section through the anterior part of the yolk region of an embryo about seven days after impregnation is depicted. The hepatic diver-

[1] Embryologists differ as to whether the blastopore becomes the anus of the adult or closes up, a new anus being subsequently formed.

ticulum is seen cut across as it is curving forwards in the midst of the yolk cells. In a section, about four or five *behind* that shown in fig. 4, the continuity of the gut with the hepatic diverticulum will be seen. Shortly after this stage, the rudiments of the heart and pericardium have made their appearance; they are found on the ventral side of the fore-gut, immediately in front of the anterior part of the yolk. The anterior part of the yolk, although perfectly continuous posteriorly with the rest of the yolk cells, soon begins to be more definitely marked off from the hypoblast above it, by the gradual closure of the lateral and ventral walls of this part of the gut. When this condition is reached, the anterior part of the yolk constitutes a forwardly directed mass, attached by a short mesentery to the gut above. This mesentery has meanwhile been produced from the splanchnic mesoblast, as the gut became more and more separated from the yolk. This anterior part of the yolk is also surrounded by a definite capsule of splanchnopleure, and is ventrally attached to the body wall below, where the somatopleure and splanchnopleure are continuous. In this constricted off portion of the yolk, the primary hepatic diverticulum lies. These points are shown in Plate IV. fig. 5, which represents a section of a frog's larva, about twelve days from impregnation, *i.e.*, about four days after hatching, and taken about fifteen sections behind the posterior limit of the heart, and just in front of the point of junction of the hepatic diverticulum and the gut. The anterior portion of the yolk above described is important, because it becomes bodily converted into the liver in the course of subsequent development, *the yolk cells being directly transformed into liver tissue.*

The steps in this conversion of yolk into liver substance can readily be traced. The first step is seen on a series of sections of a frog's larva about thirteen days after impregnation. By this time the heart, which lies immediately in front of the anterior limit of the yolk, is a fairly well-developed structure, and has already become twisted on itself, and distinguished into a dorsal or venous, and a ventral or arterial portion. By examining every section in order, behind the level of the heart, we are able to trace the venous blood-vessels from their

entrance into the sinus venosus backwards to their origin. The venous end of the heart is found to become continuous with a large vein lying in the splanchnopleure between the gut and the anterior part of the yolk. It can be traced through about ten sections, and then becomes resolved into a number of blood lacunæ lying in the midst of the localised anterior part of the yolk above referred to. These lacunæ *have no definite walls*, although a marked epithelium of flattened cells can be seen in the cavities of the heart, and can be traced along the venous trunk for some distance. The lacunæ just described are shown in Plate IV. fig. 6, which is a drawing of a section about the eighteenth behind the middle of the cardiac region. Continuing the examination, in order of the sections backwards, we find that the lacunæ are gradually lost, none whatever being found behind the junction of the primary hepatic outgrowth with the gut. In the post-hepatic region the mesenteron is still incomplete, and is quite a narrow tube, whose dorsal wall only is formed of true hypoblast. As yet there is no constriction of the anterior part of the yolk from the post-hepatic portion.

The results of the continued excavation of the hepatic part of the yolk by blood lacunæ can be studied in sections of a frog's tadpole, about six or seven days after hatching. A section taken in front of the primary hepatic diverticulum of a larva of this age, will show that the breaking up of the anterior part of the yolk by blood spaces is now so marked that a network of anastomosing cylinders of cells is found interlacing with a network of blood spaces, and taking the place of the orginally solid mass of yolk cells. This deeply excavated condition of the hepatic part of the yolk is found only in front of the primary liver outgrowth. The strands of the network of what may now be called liver substance are at this stage solid, and several layers of cells deep. I cannot, at this stage, find any trace of a wall to the blood spaces, which are therefore lacunæ, lying in the midst of a general parenchyma. These appearances are shown in figs. 7 and 8. Fig. 7 is a drawing of a section under a low power, taken about eight sections behind the posterior limit of the heart. Fig. 8 represents a portion of the liver from the same section, seen under a high power.

The further progress of the development of the proper liver

substance from these transformed yolk cells can easily be traced in sections of tadpoles of different ages. Soon a condition is reached which can with certainty be identified as practically adult liver. When the transformation of yolk cells into liver tissue has once begun, the tissue of the young organ begins to grow by cell division, which takes place chiefly in its peripheral parts, and also by extension of the cell cylinders. The organ thus grows so as to attain considerable magnitude. By the deeper and deeper excavation of the substance of the organ by blood channels, and by the gradual tunnelling of the cell cylinders so as to convert them into an anastomosing network

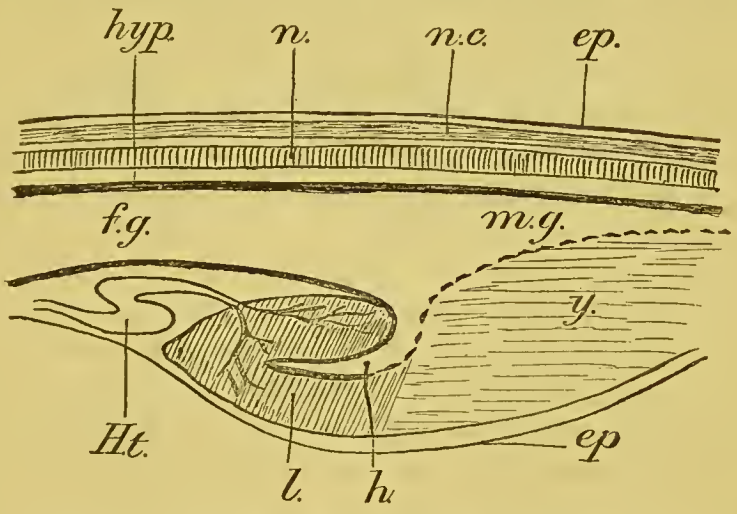


Fig. 10.—Diagram of a longitudinal section of a frog's tadpole. *Ht.*, the heart; *f.g.*, fore-gut; *hyp.*, hypoblast; *n.*, notochord; *n.c.*, nerve cord; *ep.*, epiblast; *m.g.*, mid-gut; *h.*, primitive hepatic diverticulum; *l.*, the portion of the yolk which becomes converted into liver; *y.*, the post-hepatic part of the yolk.

of tubes, together with the arrangement of the cells composing the cylinders, into a single layer around a central lumen, the characteristic features of the adult organ are acquired. A stage in this process is shown in Plate IV. fig. 9. At an early stage in the formation of the liver tubes, *i.e.*, when the cell cylinders are first penetrated by "secretion" channels, the lumina of the tubes are much more distinct than they are in the adult liver. Fig. 10 is a diagram of a longitudinal section of a frog embryo, showing the relations of the heart, alimentary canal, primary hepatic diverticulum, and yolk substance.

2. *Development of the Liver in the Chick.*—The first rudiment of the liver of the Chick is found in sections of a chick's embryo of about 55–60 hours' incubation. It consists of a cæcal diverticulum of the hypoblast growing forwards, mainly dorsal to the common vitelline vein close to its junction with the venous end of the heart. At its origin, the diverticulum lies between the two vitelline veins just prior to their junction into the common vessel (afterwards *ductus venosus*) which joins the heart. As is well known, the point of divergence of the vitelline veins at this stage marks the posterior limit of the closure off of the anterior *cul de sac* of the gut from the hypoblastic covering of the yolk sack; and the primary hepatic diverticulum really arises at this posterior limit of the fore-gut, and in such a situation that it is impossible to say whether it is a diverticulum of the gut or of the yolk sack itself. As seen in a transverse section of an embryo of this age (55 hours), the primary hepatic diverticulum is found to be compressed dorso-ventrally, and spread out somewhat laterally. A second diverticulum, arising from the hypoblast at the same point as the other, is present at this stage. This second outgrowth lies to the ventral side of the common vitelline vein, and is not so marked a structure as the dorsal diverticulum; nor does it extend so far forwards. Both diverticula are contained in the loose splanchnic mesoblast which lies just behind the heart, and are separated from the blood in the vitelline vein only by the thin epithelial lining of the latter. The two diverticula are clearly branches of one common outgrowth, the common portion lying in the angle between the two vitelline veins at their junction into a common trunk (Plate IV. fig. 11). Shortly after the formation of the primary hepatic outgrowth, the hypoblastic cells of it begin to proliferate, growing chiefly from the sides of the dorso-ventrally compressed portion. The proliferation of the hypoblast cells takes the form of solid outgrowths lying in the midst of loose splanchnopleure cells, and tending to grow around the common vitelline vein. At the same time, there take place inruptions, as it were, of capillary blood-vessels from the vitelline vein into the solid mass of proliferated hypoblast, breaking it up into more or less branched rods of

cells. Very soon the distinction between the dorsal and ventral parts of the original diverticulum is lost, and the cells produced by proliferation of the hypoblast of the two portions of the diverticulum become continuous with each other around the vitelline vein. These points can be well seen in sections of chick embryos of about 70 hours' incubation (Plate IV. fig. 12).

The production of blood capillaries in the midst of the hepatic hypoblast rapidly extends, and is so marked a feature that it is difficult to say whether the solid hepatic mass becomes broken up by the formation of blood-vessels in it, or whether cylindrical outgrowths of cells from the original diverticulum are taking place, and are splitting up the vitelline vein itself. From the descriptions ordinarily given in the works on embryology, the latter would appear to be the nature of the process. In my sections, however, I can find no definite evidence that this is so. It seems to me that the process of proliferation of hypoblast cells at the sides and extremity of the hepatic outgrowth, and the production of capillary blood-vessels by differentiation of mesoblast cells, are occurring at one and the same time, the real nature of the process being a breaking up or honey-combing of a solid mass of cells in a manner similar to the tunnelling of the yolk cells in the frog. At any rate, there is soon formed a network of solid cylinders of cells with large blood capillaries or sinuses, occupying the meshes of the network.

Very soon, *e.g.*, in an embryo of 75 hours' incubation, the line of closure off of the fore-gut from the yolk sack has extended further back, and the primary hepatic outgrowth is now found to undoubtedly arise from the gut, and not from the indifferent region between closed gut and yolk sack, as was the case at the earlier stage already described.

The process of production of hepatic cylinders goes on fairly quickly, and by about 85 hours' incubation, the solid cell cylinders forming a network around the vitelline vein can be well seen. The blood in the vitelline vein and its capillary offshoots is only separated at this stage from the cells of the hepatic cylinders by a single layer of flattened epithelium, the walls of the vein and its capillaries being alike in this

respect. The cell cylinders are at this stage composed, in some places of two, but mostly of three or four layers of cells. By about 90 hours' incubation, the production of cell cylinders has much advanced, and the general appearance of the organ under a low power is, at first sight, very like that found in the adult state of the liver of a fish. Under a high power, moreover, the cells of the hepatic cylinders, in some parts, have begun to be arranged in a single layer around a lumen. In other parts, notably the peripheral portions of a section, the proliferation of the hepatic hypoblast is still taking place, and the cell cylinders here are still solid, and, in some places, form masses of cells several deep, and as yet not excavated by blood capillaries. The appearances found in a section at this stage, therefore support the view above expressed that a solid mass of hypoblast is really split up by the formation of blood channels in it (Plate IV. fig. 13).

As to whether the cell cylinders are originally solid or hollow there is a difference of opinion amongst observers. Balfour found that they are hollow at first in Elasmobranch fishes, in Amphibia, and in some Mammals. Remak and Kölliker both described them as originally solid in Aves. Kölliker also found them to be solid in the Rabbit. I am quite satisfied from my specimens that they are originally solid in the Chick, and also in the Frog. The extent to which the primitive hepatic diverticulum itself contributes to the formation of liver substance is clearly small. Although it branches somewhat, yet it penetrates but slightly into the developing organ, whose proper tissue evidently results from a proliferation at the extremity of the diverticulum. The proper liver substance is obviously not produced by any extensive branching of the original diverticulum as a tube, whose walls might form hepatic cells. It is quite easy to distinguish the primary hepatic diverticulum and the hepatic cylinders from each other in a section of, say, a 96 hours' Chick, for not only are the lumina of the diverticulum and its branches very large, as compared with those in the developing proper tissue, but the characters of the cells forming the walls of the two structures are different.

The facts in the development of the liver of the Chick here

summarised completely establish the opinion which Lewis Jones and I arrived at in our paper on the vertebrate's liver, already alluded to. We suggested there that the primitive hepatic diverticula alone have developed into bile ducts, and that the solid cell mass produced at its extremity, and from which the proper liver tissue is developed, is to a great extent distinct from it. It is clear also that the minute biliary channels in the hepatic cylinders are not produced by an extension of the lumen of the primitive diverticula, but by the secondary formation of more minute channels in the midst of solid rods of cells. The same facts are found in the young liver of the frog's tadpole—the extent to which branches of the primitive diverticula have extended into the organ to form fine ducts is but very limited.

The minute characters of the liver, whose early development in the Chick is thus briefly described, undergo but little change in the later stages of incubation. The texture of the organ, however, becomes more dense, and the network of hepatic tubules less easy to recognise. The network, however, always remains recognisable in well-stained specimens even to adult life. A figure of the appearances presented in a section of the liver of a Chick just hatched is given in the *Journal of Physiology*, vol. x., plate xxviii., to which the reader is referred. One fact as to the appearance of the liver in the later stages of incubation must, however, be particularly noticed. It gradually acquires the same general colour and appearance as the yolk of the egg, and except that it is more dense might by a careless observer be mistaken for it on naked-eye examination. I have not yet had time to examine into the nature of the pigment which gives the liver of the later portions of incubation this appearance, but I have little doubt that the pigment is the same which is present in the yolk, and that it has been transferred to the liver from the yolk in the course of development. The liver cells in a just hatched Chick, moreover, are deeply excavated by spaces for oil drops. These facts must clearly have some importance in connection with the question of the functions of the liver during embryonic life.

3. *Development of the Liver in Mammals.*—As to this part of the subject I have at present only a few scattered observations on the livers of rabbit's, pig's, and cat's embryos.

In a cat's embryo about 5 mm. in length, I found that the production of a network of liver cylinders has already taken place. These cylinders are, however, solid, not having yet become tunnelled by the production of biliary channels. The cells of the cylinders, moreover, as yet show no sign of an arrangement into a single layer; the cylinders are, in fact, several layers of cells in thickness, and show no signs of having been formed by anastomosis of primitively distinct rods. The whole appearance is suggestive of an originally solid mass having been broken up by the production of blood spaces in it. The blood spaces have a wall of a single layer of epithelium, which can be traced by the elongated, oval, deeply-stained nuclei, at the margins of the blood spaces, and between them and the masses of liver cells. A good idea of these appearances may be obtained by inspection of Plate IV., fig. 14.

In a section through the liver of a cat's embryo 15 mm. in length, the production of proper hepatic tissue has considerably progressed from that above described. It now has a striking resemblance to the liver of an adult fish, *e.g.*, the eel. It is composed of a network of anastomosing tubules measuring about 30μ across. Where a strand of the network has been cut transversely, the tubule is found to consist of a single layer of cells, arranged around a small central lumen, about five cells being required to complete the circle of the section. The nuclei are distinct, and lie in about the centres of the cells. The whole arrangement is very irregular, and the blood spaces occupying the meshes of the network are large (Plate IV., fig. 15). The walls of the blood spaces are well formed, and there is as yet no trace of a lobule.

Although the two embryos whose livers are here briefly described are, so far, isolated specimens, yet, taken together, they indicate that the liver of the Cat is developed in much the same way as that of the Frog and Chick, viz., by the production of a network of solid rods from a primitively continuous mass by the development of blood-vessels in it, and the subsequent secondary formation of biliary lumina in the solid cylinders.

D. *Theoretical Considerations.*

The account of the development of the liver in Vertebrata

given in the foregoing pages, is, I think, sufficient to justify the following conclusions:—

1. The liver, at the time of its primary origin, and also throughout development, is intimately connected with the yolk or with the yolk sack. In some cases, *e.g.*, the Frog, yolk cells are actually converted into liver substance.

2. It has a very intimate association with the blood-vessels which carry blood from the yolk sack, or its equivalent, to the heart.

3. The liver begins to be developed at a time when the absorption of the yolk and its utilisation for the supply of food to the growing embryonic cells is beginning to be particularly active.

4. The liver is primitively a simple saccular outgrowth from the alimentary canal, and is preserved in this state in the adult of one Chordate only, viz., the *Amphioxus*. This clearly represents the first step in its evolution.

5. The cæcal pouch thus evolved does not become drawn out into saccular prolongations as in invertebrates, but gives rise to a solid mass of cells at its extremity.

6. The solid mass of cells produced in this way becomes the proper liver tissue of all those Chordates which possess anything more than a simple outgrowth (all those higher than *Amphioxus*).

7. All the types, in which a solid mass of liver cells is produced, have also a certain amount of yolk substance.

8. The originally solid mass of hepatic cells early becomes broken up by the production in it of blood capillaries connected with, or in the course of, the veins of the yolk sack.

9. The minute "biliary" canals of the liver substance are formed subsequently by the hepatic cells arranging themselves around central lumina.

10. These "biliary" channels are not formed by the extension of the original diverticulum of the gut, but separately, and subsequently open into the original outgrowth, as it were, into a common duct.

These conclusions seem to me to clearly indicate that the proper liver tissue is first evolved as an embryonic organ of nutrition, and for the purpose of producing some change in yolk

substanee to fit it for use as an immediate formative material in the metabolism and growth of embryonic eells and tissues. I should imagine that the erude food yolk—a more or less solid material—beeomes liquified by the aetion of the hypoblastic eells of the yolk sack (or by a ferment produeed by them), and is then absorbed and earried by the veins of the yolk sack towards the heart. In its eourse, this absorbed yolk substance is earried in the blood of the yolk veins through the eapillary vessels of the young liver, whose cells, I imagine, further act upon it, and elaborate it into some substanee more immediately fitted to be utilised for the eonstruction of new protoplasm by the general embryonie eells. It is, I think, generally held by physiologists that the liver of the adult performs some such funetions, elaborating or otherwise aeting upon the absorbed produets of digestion brought to it by the "portal" system. It is eertain that it aets in this way in eonstructing glyeogen from the sugars of digestion, and it is probable that it behaves similarly upon the proteids of digestion (whether they are brought to it as peptones, or, as is more probably the case, in the form of an albumin, into which peptone is converted during absorption). For purposes of eomparison of the embryonic alimentary system with that of the adult, we may liken the hypoblastie surface, eovering and adjaeent to the yolk, to the endodermal digestive surfaee of the adult alimentary canal. From it, "digested" food is absorbed into the blood of the yolk veins as it is into the radieles of the adult "portal" system. The veins of the yolk sack behave with respeet to the liver just as the "portal vein" in the adult does. In faet, the vitelline veins of the embryo might quite appropriately be called the "embryonic portal system." I see no reason to doubt that the embryonie liver acts on the crude food material in the blood of the yolk veins just as the liver does on that of the "portal" blood of the adult—in fact, the latter would seem to be but a continuation into adult life of funetions performed by the same organ in the embryo. These eonsiderations are not only eonsistent with all that is known of the development of the liver, but also serve as a highly probable explanation of some otherwise inexplicable faets as to its origin and structure, and of the curious relations to the blood system whieh this organ has in the embryo.

Probably, at first, all the hypoblastic cells, not only those which will form the walls of the gut, but also those immediately covering the yolk contained in the yolk sack, have the power of producing all the changes in the food yolk which are necessary to fit it for use by the developing cells of the embryo. Later on, as the embryo increases, and its cells begin to become more specialised, probably the function of rendering the yolk suitable for use becomes divided into two parts, on the principle of physiological division of labour. One portion of this function, viz., "digestion," and absorption of yolk into the blood capillaries of the *area vasculosa*, continues to be performed by the general hypoblastic surface, whilst the further elaboration of the absorbed yolk substance becomes relegated to a specialised part of the hypoblastic surface, which grows out as a diverticulum from the rest (the primitive hepatic outgrowth). Then, to bring the blood of the yolk veins into closer relations with these specialised hypoblastic cells, a proliferation of the cells at the end of this diverticulum takes place, and it at the same time becomes penetrated by blood capillaries. Thus the process of "digestion" of the food yolk becomes split up into two steps, comparable to the "exterior digestion," and the "interior" or "interstitial digestion" of Claude Bernard.[1] In this summary we have an explanation of—(1) The curious relations of the liver to the blood-vessels of the yolk sack; (2) the peculiar nature of the liver tissue itself, viz., *a network of, at first, solid cell cylinders without membranous coverings or basement membranes interlacing with a network of blood capillaries*; (3) the very large development of the liver in the embryo, when such an organ can have no "glandular" functions of any importance; (4) the fact that in some animals the proper liver tissue is actually developed by transformation of yolk cells; (5) the fact that, in later stages of incubation in the Chick, the liver becomes impregnated with yolk pigment.

Not only does the liver act as the elaborator of the crude yolk in the embryo, but it probably also, particularly in the later stages of embryonic life, becomes a storehouse of reserve substance, which is being absorbed faster than it is needed by the developing tissues, and so fat, glycogen, and possibly reserve

[1] *Leçons sur les phénomènes de la vie*, t. ii., 1879.

proteid substance, becomes stored in it for future use. A continuation of this function into adult life is seen in the "glycogenic" function of the fully-formed organ, and it is not improbable that the liver of the adult is a storehouse of reserve proteid substance also.

We have now to explain how the solid hepatic network comes subsequently to be a network of tubules with fine lumina draining into the hepatic duct or primary diverticulum. A little reflection will make it clear that, if the liver cells are in the embryo actively metabolic, producing an elaborated food substance, there would probably be formed also some waste materials, which not only are of no further use, but which must be got rid of or excreted. This end could quite easily be attained by the liver cells arranging themselves in rows around central drainage channels, penetrating the axes of the cell cylinders. Into the minute drainage channels thus constituted the waste or degradation products of the hepatic metabolic processes could easily be discharged, and, making their way to the primitive diverticulum, could, for the time, be got rid of. I believe this to be the original nature of the "biliary" secretion channels; and that some such process is occurring in fœtal life is evidenced by the well-known "meconium" of the fœtus. The production of this substance is merely an accidental accompaniment of the performance of the proper hepatic functions of the embryo. The same production and excretion of waste useless material is continued into adult life in the formation of bile. Of all the fluids poured into the alimentary canal, in the adult, the bile is the only one which contains no digestive ferment, and has no digestive properties, beyond the purely mechanical one of assisting in the emulsification of fat.

It will be seen from the foregoing that I am distinctly of opinion that the liver is, primarily and essentially, an organ of nutrition, and that bile formation is only quite a secondary and subsidiary function of it. Perhaps, however, it is a question whether the earliest step of all in the origin of the vertebrate's liver, viz., the stage which remains permanently in *Amphioxus*, does not represent an organ of "secretion" rather than an organ of nutrition. Other than the saecular "liver," the *Amphioxus* has no "gland" in relation to its alimentary canal, and it may,

in this animal, be of secretory function, producing a polyenzymatic fluid, and so be comparable to the simple saccular "livers" of invertebrates. Whether this be its primary nature or not in *Amphioxus*, we must not lose sight of the fact that this animal has now been shown to possess a "portal" system of veins quite comparable to that of higher vertebrates, and also of the fact that in no invertebrate has anything like a "portal" system of blood-vessels been shown to exist.

We have already been led to conclude that the vertebrate's and invertebrate's "liver" differ fundamentally in structure, and that the former was not evolved from the latter. The account of the development of the liver in the frog, chick, and mammal accentuates this conclusion; and in answer to the inquiry—How, then, was the vertebrate's liver evolved?—the facts above described and discussed seem to point to the conclusion, *that this organ primarily arose as an embryonic "organ of nutrition," evolved* pari passu *with the evolution of those vertebrates which produced large yolked ova.* We also conclude that after it has been evolved in this way, the organ persists into adult life by being adapted to perform the same functions in the adult as it does in the embryo.

Lately, the view of Gaskell,[1] that the ventricles of the brain and the spinal canal of vertebrates represent the stomach and intestine of a crustacean-like ancestor, around which the supraœsophageal and infraœsophageal ganglia, and ventral nerve chain of such an ancestor have grown, has received very considerable support from the facts recently published by him in a second paper[2] on this subject. If Gaskell's theory be correct, that a crustacean alimentary canal has become invaded by and enclosed within the central nervous system in the vertebrate, it is clear that the crustacean "liver" could not be the morphological equivalent of that of vertebrates. Some of the conclusions, therefore, at which I arrive in this paper are quite in harmony with Gaskell's theory, and form, so far as they go, a point in confirmation of his views. Gaskell, moreover, has

[1] "On the Relation between the Structure, Function, Distribution, and Origin of the Cranial Nerves, &c.," *Journal of Physiology*, vol. x. p. 153, 1889.

[2] "On the Origin of Vertebrates from a Crustacean-like Ancestor," *Quart. Jour. Mic. Sci.*, Aug. 1890.

found remnants of what he identifies as the crustacean "liver," lying outside the brain substance, within the cranial cavity of the Ammoecœtes. I regard this as a fact of very great importance, and one which, if justified, is confirmatory of the conclusion which I have independently arrived at, that the liver of vertebrates has not been evolved from the "gland of the mid-gut" of any invertebrate. I am, however, not quite satisfied, from a perusal of Gaskell's description and an inspection of his drawings of the cellular mass which he identifies as the crustacean "liver," that it has the appearances which one would expect to find in the degenerated representative of the normal crustacean "liver" which I have described in this paper; nor am I satisfied that the appearances which Gaskell has found in Ammoecœtes are those which, from my examination of invertebrate "livers," I should have expected this organ in the archi-crustacean to possess. The peculiar tissue which Gaskell describes and figures in Ammoecœtes, appears to consist of a closely packed mass of large cells, each having a nucleus surrounded by remains of a protoplasmic body. Between the cells there appear to be deeply pigmented lines, but, Gaskell says, no connective-tissue elements. Gaskell finds that "these cells are solid polygonal bodies, pressed together in all directions without any sign of being in connection with specially arranged connective-tissue elements either in one direction or the other the staining material in the cell consists of a broken-down network which starts from the nucleus and forms scanty strands which pass towards the periphery." As to the nature of the pigmented lines between the cells of this peculiar tissue, Gaskell says—"These lines and collections of pigment are the remains of the blood channels which supplied the cells of the old cephalic liver with blood." This description of Gaskell's would lead one to infer that the degenerate tissue which he describes, if liver at all, was built up on the plan which I have shown is that of the proper tissue of the vertebrate's liver, viz., a solid mass of cells penetrated with blood-vessels and with no connective-tissue elements. If it were known that the crustacean or archi-crustacean "liver" had the same plan of structure as the vertebrate liver, then Gaskell's conclusion that this tissue "is precisely what one would expect if it represented the degenerated remains of the

cephalic liver of the crustacean-like ancestor of the Ammocœtes" would, I think, be fully justified. But, on the contrary, the fundamental type of structure which I find in the "gland of the mid-gut" of crustaceans, in its simplest form, is that of a duct with loose saccular cæcal dilatations lined by epithelial cells planted on a wall of connective tissue. If Gaskell's peculiar tissue were really the remains of crustacean "liver" I should have expected to find some evidence of the collection of the cells into groups surrounded by some connective tissue to represent the cæcal pouches, the cavities of which might or might not have already been obliterated. For this reason, although I am not prepared to deny Gaskell's interpretation of the peculiar tissue of Ammocœtes, I find it difficult to unreservedly accept it.

E. *Summary.*

1. The "liver" of invertebrates is *not* morphologically the same as that of vertebrates.

2. The "liver" of invertebrates is the gland of the mid-gut, and has, when present, fundamentally the same nature in all.

3. It is composed in invertebrates of cæcal pouches, lined by secreting epithelium, surrounded by connective-tissue membranes.

4. In the Vertebrata, the liver is made up of a network of tubules, interlacing with a network of blood capillaries, and unprovided with any basement membrane separating the blood capillaries from the liver cells.

5. The invertebrate's "liver" is essentially a gland, secreting a digestive fluid containing ferments.

6. The vertebrate's liver is primarily an organ of nutrition for the embryo; and has been adapted to perform similar functions in the adult.

7. In its evolution, the vertebrate's liver is intimately associated with the absorption of the food yolk of the egg.

8. The vertebrate's liver has *not* been evolved from the "gland of the mid-gut" of any invertebrate.

9. The pancreas of the vertebrate is somewhat similar in structure and functions to the "gland of the mid-gut of invertebrates; although the question of whether these two organs are morphologically equivalent is an open question.

EXPLANATION OF PLATE IV.

Fig. 1. "Hepatic" cæca from a section of the "liver" of *Mya arenaria*. *a.*, connective tissue wall of the cæca; *b.*, deeply stained portion of the "hepatic" cells, containing nuclei; *c.*, honey-combed part of the protoplasm of the "liver" cells; *d.*, cavity of cæcum.

Fig. 2. "Hepatic" cæca from a section of the "liver" of *Astacus fluviatilis*. *a.*, connective tissue basement membrane; *b.*, "liver" cells; *c.*, cavity of the cæcum; *d.*, deeply stained inner border.

Fig. 3. Transverse section of a frog's embryo about six days after impregnation. *ep.*, epiblast; *n.c.*, nerve cord; *n.*, notochord; *hyp.*, hypoblast; *p.*, mesoblastic somite; *mes.*, mesenteron; *so.*, somatopleure; *spl.*, splanchnopleure; *y.*, yolk cells.

Fig. 4. Transverse section through the anterior part of the yolk of a frog's embryo about seven days after impregnation. *n.c.*, nerve cord; *n.*, notochord; *hyp.*, hypoblast; *mes.*, mesenteron; *y.*, yolk cells; *h.*, primary hepatic diverticulum.

Fig. 5. Transverse section of a frog's larva about twelve days after impregnation. *W.B.*, Wolffian body. Other references as in figs. 3 and 4.

Fig. 6. Transverse section through the anterior part of the yolk region of a frog's larva about thirteen days after impregnation. *b.lac.*, blood spaces in the midst of yolk cells. Other references as in preceding figures.

Fig. 7. Transverse section through the same region of a frog's larva about fifteen days after impregnation. *l.*, liver. Other references as before.

Fig. 8. A portion of the same section as seen with a high power. *b.lac.*, blood spaces in the liver; *hep*, network of liver cells.

Fig. 9. Section of the liver of a frog's tadpole about five days older than that from which figure 8 is drawn. *mes.*, intestine; *h.*, primary hepatic diverticulum, *i.e.* hepatic duct; *hep.*, network of liver tubules, each composed of a single layer of cells around a central lumen; *b.lac*, blood spaces.

Fig. 10. See page 183 of text.

Fig. 11. Transverse section of a Chick embryo of about 60 hours' incubation. *n.c.*, nerve cord; *n.*, notochord; *ao.*, aorta; *p.c.v.*, posterior cardinal vein; *so.*, somatopleure; *spl.*, splanchnopleure; *am.*, amnion; *v.v.*, common vitelline vein; *h.*, primary hepatic diverticulum; *h'.*, ventral branch of the primary hepatic diverticulum.

Fig. 12. Transverse section across the region of the developing liver of a 70 hours' Chick. *p.c.v.*, posterior cardinal vein; *ao.*, aorta; *p.p.*, pleuro-peritoneal cavity; *i.*, intestine; *h.*, primary hepatic diverticulum; *so.*, somatopleure; *spl.*, splanchnopleure; *v.v.*, vitelline vein; *am.*, amnion; *l.*, network of liver cylinders; *c.*, capillary blood-vessels of the liver.

Fig. 13. Portion of a longitudinal section of a 96 hours' Chick

embryo. *e.*, epithelial lining of the blood capillaries of the liver; *c.*, the blood spaces. Other references as in figs. 11 and 12.

Fig. 14. A portion of the liver of a cat embryo about 5 mm. in length. *a.*, solid cylinders of liver cells; *b.*, blood spaces in the solid mass dividing it into a network; *c.*, epithelial lining of the blood spaces.

Fig. 15. A similar section of the liver of a cat embryo about 15 mm. in length. *a.*, lumen of liver tubule; *b.*, liver tubule; *c.*, blood in the blood capillaries; *d.*, epithelial lining of blood capillaries.

Fig. 1.



Fig. 2.

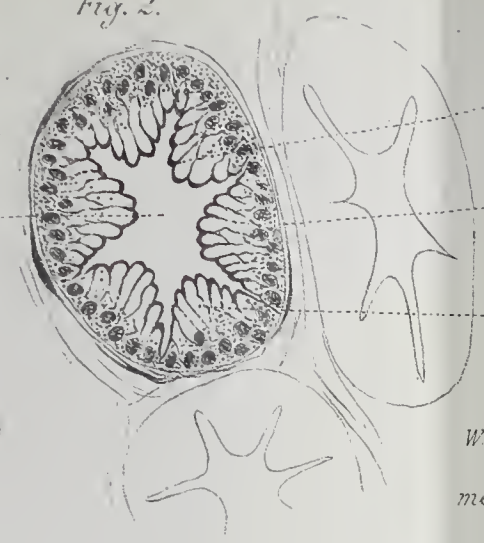


Fig. 3.

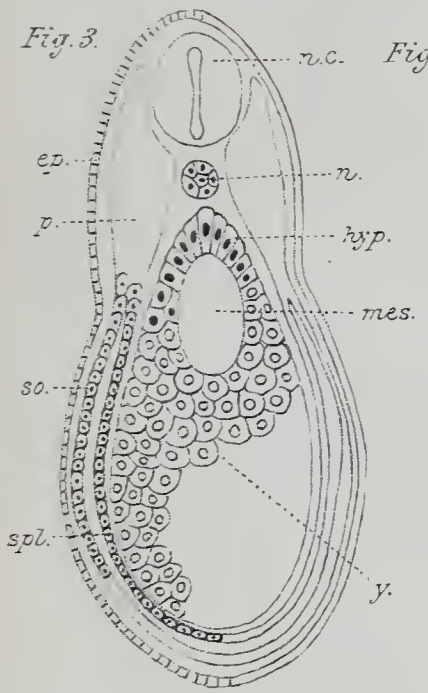


Fig. 4.

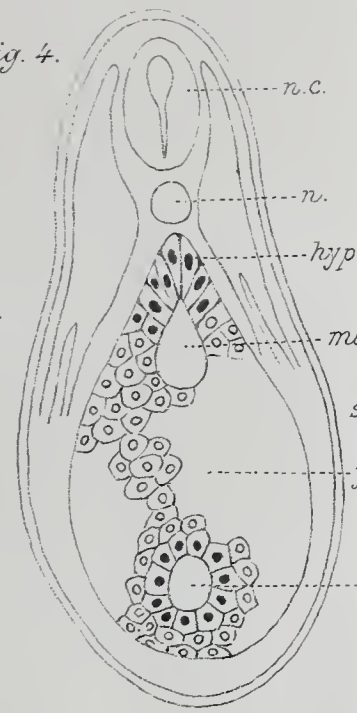


Fig. 5.

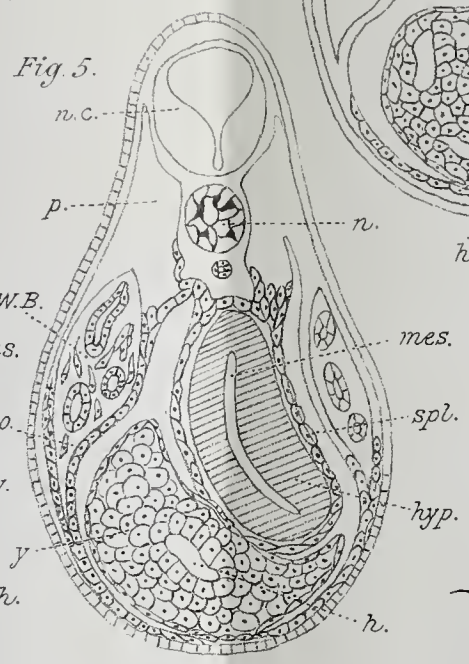


Fig. 6.

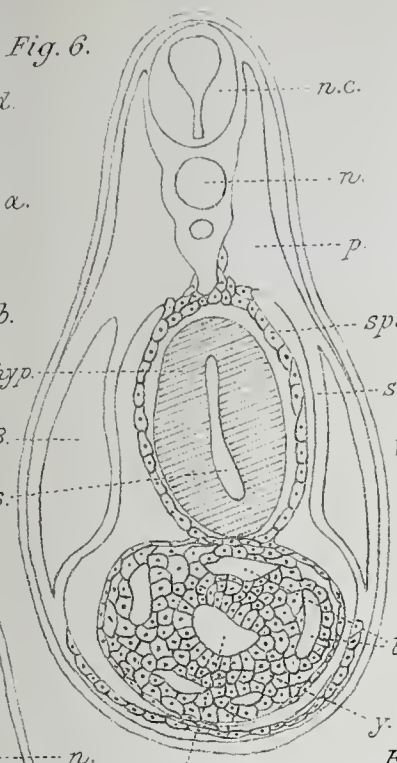


Fig. 7.

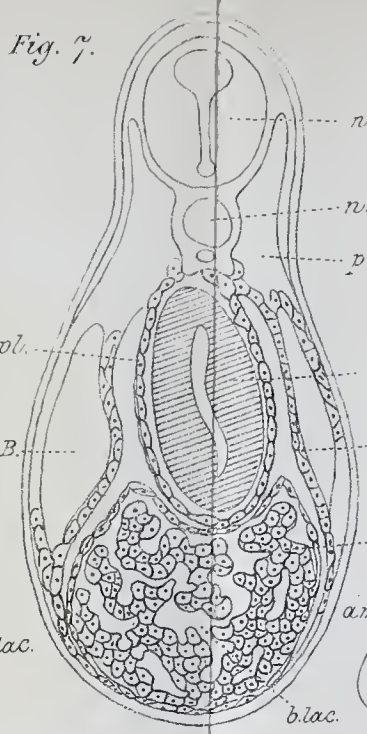


Fig. 8.

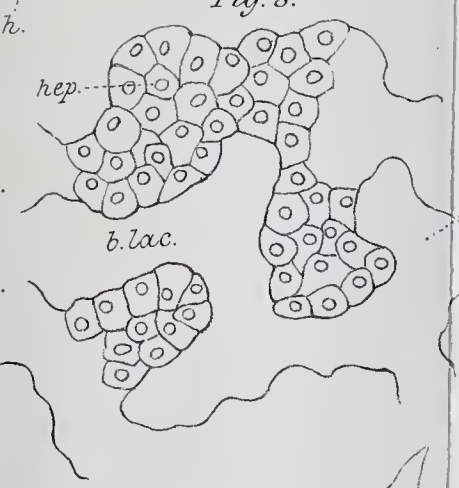


Fig. 11.

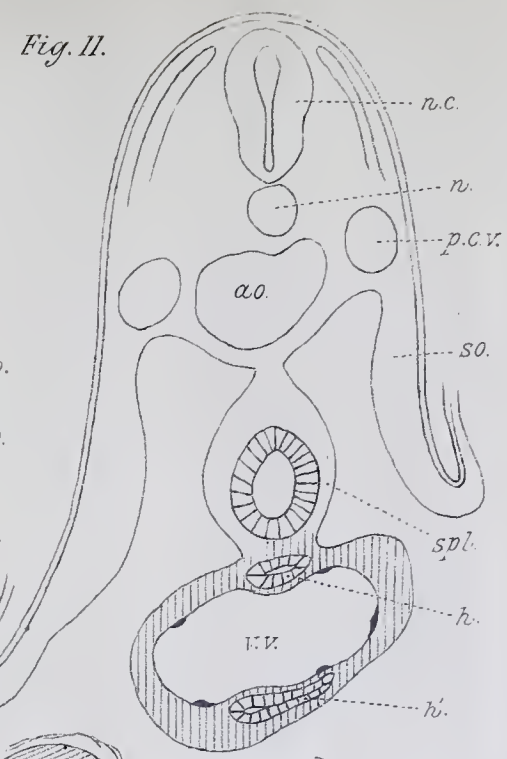


Fig. 9.

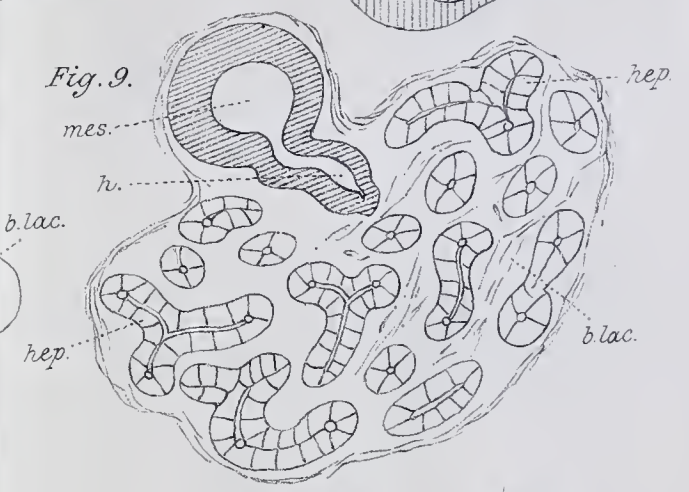


Fig. 12.

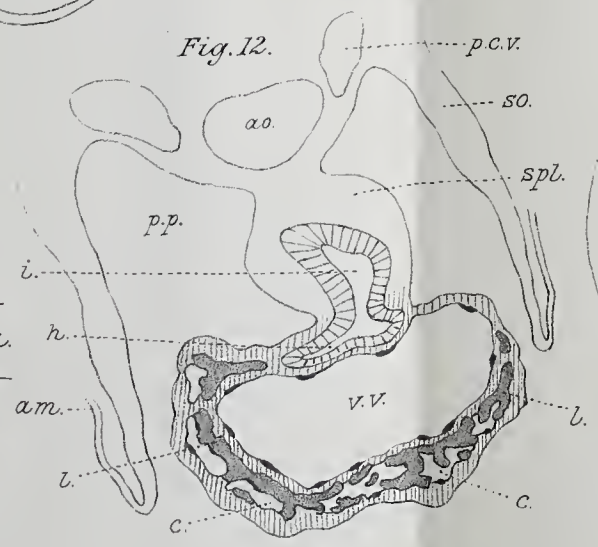


Fig. 13.

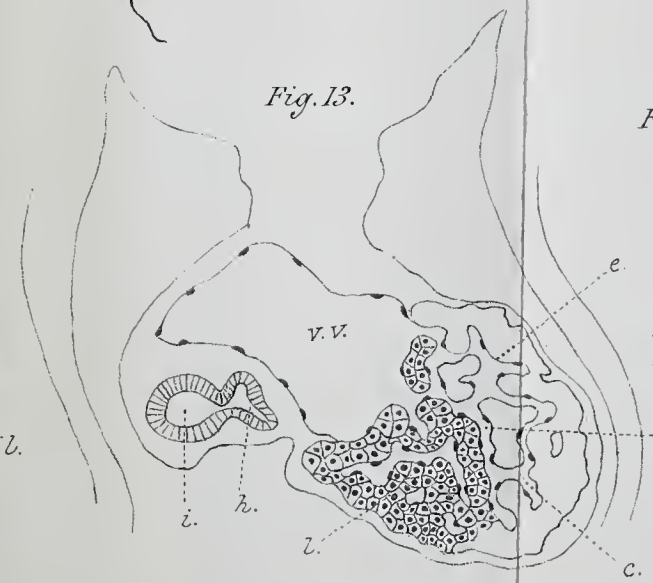


Fig. 14.

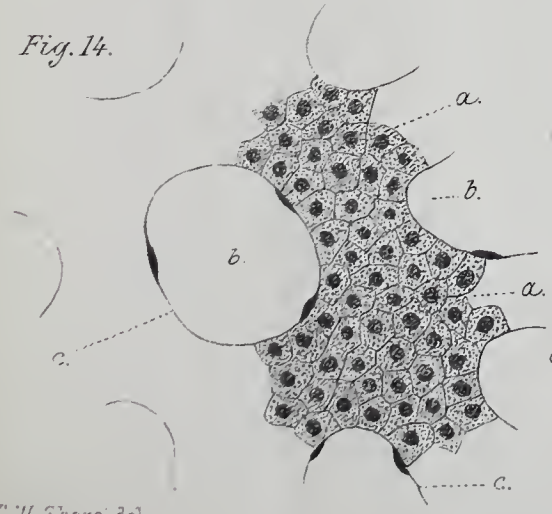
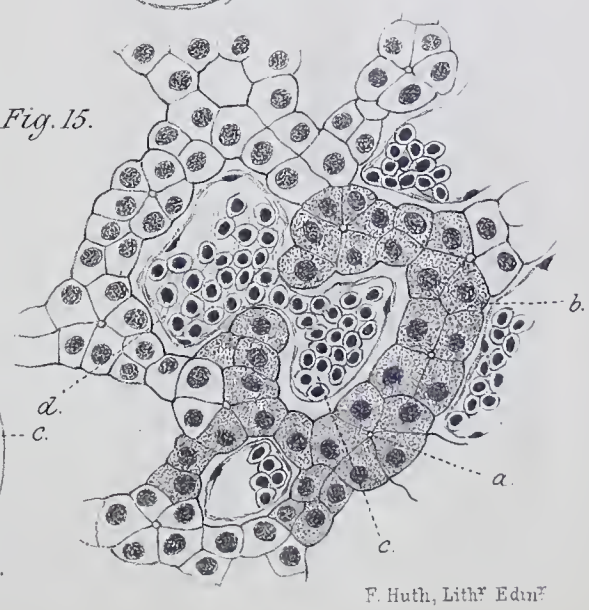


Fig. 15.



T. W. Shore del.

F. Huth, Lith.ʳ Edin.ʳ


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